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Carotenoids: Biology and Treatment

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ABSTRACT. Carotenoids are pigments found in plants and microorganisms, but not synthesized in animals. Fewer than 10% of the carotenoids can function as vitamin A precursors in mammals. Carotenoids and retinoids have chemical and metabolic similarities and differences, and some overlap in biological activities. Carotenoids in tissues reflect food choices. Carotenoids exhibit biological activities as antioxidants, affect cell growth regulation, and modulate gene expression and immune response. Epidemiologic evidence links higher carotenoid intakes and tissue concentrations with reduced cancer and cardiovascular disease risk, although results from clinical trials do not support β-carotene supplementation as a strategy to reduce risk. Continued research in this area is likely to stimulate better intervention strategies with clinical and public health applications. PHARMACOL. THER. 75(3):185–197, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Carotenoids, diet, antioxidants, vitamin A, retinoids, clinical trials.

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ABBREVIATIONS. ATBC, alpha-tocopherol beta carotene; CARET, Carotene and Retinol Efficacy Trial; HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very-low density-lipoproteins.

1. INTRODUCTION

Carotenoids are pigments that are found in plants and microorganisms, but are not synthesized in animals. Nearly 600 of these compounds have been identified in nature (Fig. 1) (Goodwin, 1986; Olson, 1989). Fewer than 10% of the carotenoids can be metabolized to retinol and function as vitamin A precursors in mammals. However, carotenoids and retinoids have some similarities in their chemistry and metabolism, and recent evidence also suggests some overlap in biological activities (Krinsky, 1994a). Human tissue contains only a fraction of the total number of carotenoids that have been identified in the food supply (Khachik *et al.*, 1991). The predominant carotenoids observed in the plasma are β-carotene, lycopene, lutein, β-cryptoxanthin, and α-carotene, which account for 90% or more of the circulating carotenoids in humans (Bieri *et al.*, 1985).

Carotenoids are known to exist in different geometric forms (*cis*- and *trans*-isomers). These forms may be interconverted by light, thermal energy, or chemical reactions (Stahl and Sies, 1993); for example, the cooking of vegetables promotes isomerization of carotenoids from the *trans* to the *cis* forms. Interest in the *cis*-isomers of the carotenoids has been stimulated by the recognition of isomer-specific biological functions that clearly exist for the retinoids (Mangelsdorf *et al.*, 1994; Norum and Blomhoff, 1992) and may exist for the carotenoids. Synthetic β-carotene is almost entirely in the *trans*-isomeric form.

Still used in clinical laboratories, the traditional approach to measuring these compounds in serum or plasma is actually a crude measure of total carotenoids, which is obtained by deproteinization, lipid extraction and spectrophotometric measurement at 450 nm, using the extinction coefficient for β-carotene (Nierenberg *et al.*, 1989). In the analysis of foods, early methods focused on quantifying only those carotenoids that were thought to be potential precursors of vitamin A, so carotenoid values in food-content databases were traditionally expressed as (and incorporated into) vitamin A values in international units or retinol equivalents (Beecher and Khachik, 1981). The introduction of HPLC to carotenoid research in the 1970s enormously enhanced the ability to study the biological characteristics and activities, because this tool enabled the separation and more accurate quantification of these diverse compounds. Because the various carotenoids exhibit

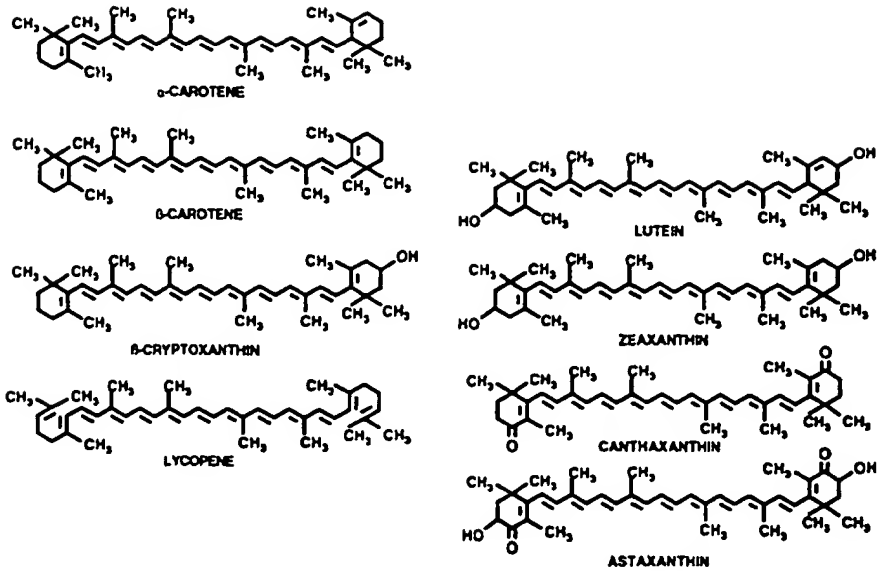

FIGURE 1. Structures of selected carotenoids. Reproduced from Krinsky (1993), with permission of the author and the copyright holder, Annual Reviews, Inc., Palo Alto.

some differences in their chemical and biological characteristics and have unique distributions in foods, examining the individual compounds is necessary when investigating their role in human health and disease.

2. ABSORPTION, TRANSPORT AND STORAGE

2.1. *Absorption*

Absorption and metabolism of carotenoids varies a great deal among animal species. In humans and only a few other mammals (i.e., some other primates, the ferret, the bovine), an appreciable amount of the carotenoids can be absorbed intact by the mucosal cells and subsequently appear unchanged in the circulation and peripheral tissues (Bowen *et al.*, 1993; Erdman *et al.*, 1993). In rodents and other white fat (versus yellow fat) animals, the provitamin A carotenoids are metabolized to vitamin A in the intestinal mucosal cells or are not absorbed (and excreted through the gastrointestinal tract) unless administered at supraphysiologic doses. Thus, plasma concentrations are normally very low and tissue distribution is not comparable with that in humans. In humans, variable proportions of the carotenoids taken up by the intestinal mucosal cells are metabolized in the process of absorption, which complicates the interpretation of plasma carotenoid response as an indicator of uptake after administration, when this approach is used in investigations (Parker, 1996). However, the materials and methods that allow stable isotope tracer studies to be conducted in human subjects were developed only recently, and results from these studies are still very limited in quantity and scope (Novotny *et al.*, 1995; Parker, 1996; Parker *et al.*, 1993).

Absorption of carotenoids takes place in the intestinal mucosa, and uptake of these compounds by duodenal mucosal cells appears to be by passive diffusion, with a concentration gradient between the amount of carotenoid in the mixed micelle and that in the cell membrane presumed to be the determinant of the rate of diffusion of these compounds (Parker, 1996). The possibility that a carotenoid-binding cytosolic protein might play some role in the intracellular transport of these compounds in the intestine or liver has been hypothesized, but efforts to identify or isolate such a cellular carotenoid-binding protein have not been successful. After passive uptake by the enterocyte, unmetabolized carotenoids are incorporated into chylomicra and secreted by the lymph, followed by liver uptake and release back into the circulation in association with very-low-density lipoproteins (VLDL) and ultimately in association with circulating low-density lipoproteins (LDL) (Erdman *et al.*, 1993). Two peaks are characteristically observed in the plasma after the administration of β-carotene, the first peak representing a transient increase in chylomicron-associated β-carotene (at approximately 8 hr after an oral dose), followed by a large increase (at approximately 24–48 hr), representing an increase in the LDL-associated β-carotene. Some differences in the kinetic pattern after administration of the different carotenoids have been observed (Olson, 1994). Retinol produced in the enterocyte from provitamin A carotenoids is secreted in lymph chylomicra as retinyl esters, whereas retinoic acid and other polar metabolites exit the intestinal tissue through the portal circulation.

Numerous factors have been shown to affect the absorption of carotenoids, as defined by the appearance of these compounds or their metabolites in the lymphatic or portal circulation or by an improvement in vitamin A status in those species capable of utilizing carotenoids as vitamin A precursors. Table 1 summarizes the major factors for which evidence currently suggests an effect on absorption or utilization. The efficiency of carotenoid absorption is typically relatively low overall (<30%), and the percentage absorbed decreases markedly with increasing intake (Olson, 1994).

Among the dietary factors, fat appears to exert the greatest effect on absorption. An early study of subjects consum-

TABLE 1. Factors Affecting Carotenoid Absorption and Effects Observed

Dietary factors:
Fat increases absorption
Soluble fiber (e.g., pectin) interferes with uptake
Dosage of carotenoid administered (reduced efficiency at higher levels)
Competitive interactions between carotenoids
Food form:
Location in plant tissues (e.g., plant cell chloroplasts may be less bioavailabable than chromoplasts)
Mild heat treatment increases bioavailability
Reduced particle size (i.e., blenderizing) improves extractability
Biochemical and metabolic factors:
Isomeric form, but effect (positive versus negative) varies with carotenoid
Large interindividual variability, likely due to metabolic or absorption polymorphism
Subject characteristics:
Intestinal parasites associated with reduced absorption
Malabsorption syndromes (especially involving fat) reduce absorption
Vitamin A status (due to effect on intestinal conversion)
Increased gastric pH associated with suppressed blood response

Data from Rock and Swendseid (1992), Bowen *et al.* (1993), Erdman *et al.* (1993), Parker (1996), Rock *et al.* (1996), and Tang *et al.* (1996).

ing a baseline diet providing 7% energy from fat showed that the addition of 20 mL of olive oil to the daily feeding of carrots improved the apparent uptake of β-carotene approximately 5-fold (Roels *et al.*, 1958), and, more recently, plasma response curves following β-carotene supplementation in subjects consuming very low fat (6 g) versus higher-fat (60–70 g) diets demonstrated a similarly marked difference (Dimitrov *et al.*, 1988). Across the range of fat intakes in typical Western diets (which usually provide 20–40% energy from fat), the relation between carotenoid intakes and tissue concentrations does not appear to be affected by level of fat in the diet, suggesting a threshold effect (Rock *et al.*, 1997).

One explanation for the enhancing effect of dietary fat is that carotenoids are absorbed only in the presence of conjugated bile salts, and fat stimulates the secretion of bile salts. *In vitro* studies have also demonstrated that the absorption of carotenoids involves specific requirements for micelle size and nature of the dispersion to permit uptake by intestinal cells (Olson, 1994). Dietary factors that may disrupt micelle formation or interfere with micelle contact with the mucosal cells, such as soluble fibers (e.g., pectin), have an adverse effect on carotenoid absorption (Rock and Swendseid, 1992). Dietary vitamin E has been observed to have a protective effect on carotenoid availability or utilization in studies utilizing laboratory animals (Wang *et al.*, 1995). In human studies, observations from some supplement trials have suggested the possibility of an adverse interaction between β-carotene and α-tocopherol at the level of absorption (Mobarhan *et al.*, 1994; Xu *et al.*, 1992), although this possibility has not been confirmed by results from other trials (Goodman *et al.*, 1994; Nierenberg *et al.*, 1994). Indications of absorption interactions between the different carotenoids have been evident in several studies. For example, β-carotene supplementation has been associated with a decline in plasma lutein concentrations (Kostic *et al.*, 1995; Micozzi *et al.*, 1992), and has been shown to interfere with the intestinal absorption of canthaxanthin on the basis of plasma response after administration (White *et al.*, 1993).

The location of the carotenoid pigment in the plant food source and other characteristics of the matrix in which the carotenoids are delivered also exert an effect on absorption. Improved absorption of carotenoids has been observed with carotenoid-containing foods that have been cooked (compared with raw foods) and with blenderized (versus whole) foods (Poor *et al.*, 1993; Rao and Rao, 1970). Carotenoids located in the pigment-protein complexes of cell chloroplasts have been hypothesized to be less bioavailable than those in the chromoplasts (de Pee *et al.*, 1995), although this remains to be demonstrated in humans. Heating tomato juice increases the proportion of *cis*-isomers of lycopene present and is associated with an apparent increase in total absorption of this carotenoid (Stahl and Sies, 1992). On the basis of plasma response, *cis*-isomers of β-carotene have been suggested to be less bioavailable than all-*trans*-β-carotene (Gaziano *et al.*, 1995). However, isomerization of 9-*cis*- to all-*trans*-β-carotene was recently shown to take place in human intestinal mucosa at physiologic doses (You *et al.*, 1996). Conversion in the course of the absorption process may make the isomeric form of β-carotene that is provided less crucial an issue in the overall utilization of carotenoids in the human biological system.

Interindividual variation in plasma response to carotenoid supplements or carotenoid-containing foods is appreciable, with some subjects exhibiting a large increase in plasma concentration and others exhibiting very little change in response to an identical dose (Micozzi *et al.*, 1992). Differences in rates of conversion of β-carotene into vitamin A at the intestinal level have been observed even in well-nourished individuals (Parker, 1996), which explains some (but not all) of the variability in plasma response after the administration of β-carotene. Other aspects of absorption and metabolic polymorphism are likely to exist. The presence of intestinal parasites and malabsorption associated with overall malnutrition promote impaired carotenoid absorption or utilization, which is of clinical importance when increased intake of carotenoid-rich foods is used as a strategy to treat vitamin A deficiency in economically disadvantaged groups (de Pee *et al.*, 1995).

2.2. *Transport*

Carotenoids in the circulation are transported in association with the lipoproteins, with a distribution similar to that of cholesterol, so plasma cholesterol concentrations are highly correlated with (and predictive of) circulating carotenoid concentrations when population data are examined (Clevidence and Bieri, 1993; Olson, 1994). Overall, approximately 75% of plasma carotenoids are with LDL, and the remainder are distributed between VLDL and high-density

lipoproteins (HDL), although the different carotenoids have somewhat distinctive patterns of distribution. The more nonpolar carotenoids (e.g., β-carotene, α-carotene, lycopene) are predominantly with LDL, whereas the more polar carotenoids (e.g., lutein) associate more with HDL than with LDL. Compared with α-tocopherol, which also associates with lipoproteins, the amount of carotenoids circulating with the lipoproteins is relatively small. For example, in one recent study (Romanchik *et al.*, 1995) approximately 4 carotenoid molecules were found to be associated with each VLDL and 1 with each LDL particle, whereas only 25 of every 1000 HDL particles contained carotenoid.

In population studies, circulating carotenoid concentrations are typically lower in smokers than in nonsmokers, attributable in part to the depletion of these compounds by components of cigarette smoke (Handelman *et al.*, 1996), although dietary carotenoid intake is also often lower in smokers versus nonsmokers (Brady *et al.*, 1996; Stryker *et al.*, 1988). Alcohol intake has been inversely correlated with plasma carotenoid concentrations in men, which may also be explained in part by differences in dietary intake (Brady *et al.*, 1996; Stryker *et al.*, 1988). An inverse relation between body mass and plasma concentrations of carotenoids, irrespective of dietary carotenoid intake, has been observed in both epidemiological and clinical studies (Rock and Swendseid, 1993). When confounding variables (i.e., dietary intake, plasma-lipid concentration, body mass) are considered, aging has not been shown to exert an independent effect on circulating carotenoid concentrations.

2.3. *Storage*

Adipose tissue (particularly because of its volume) and liver are the major tissue storage depots for the carotenoids, although these compounds have also been identified in lungs, kidney, cervix, prostate and most other tissues (Schmitz *et al.*, 1991). High concentrations of carotenoids are found in tissues that are rich in LDL receptors, such as the corpus luteum, adrenal tissue and testes, probably resulting from nonspecific uptake by lipoproteins. An overall correlation between the pattern of carotenoids in the circulating pool and the peripheral tissues is observed, with some notable exceptions. The macula of the eye is particularly rich in lutein and zeaxanthin, but not other carotenoids (Handelman *et al.*, 1992), and the concentration of these two compounds is roughly equal in this tissue. This ratio differs markedly from the pattern in the circulating pool, in which the concentration of lutein usually greatly exceeds that of zeaxanthin. Although carotenoids have been found in the pineal gland, none are present in the brain stem (Olson, 1994). Differences in the proportionate distribution among the isomeric forms have been observed when plasma and peripheral tissue concentrations of β-carotene are compared: the amount of *trans*- greatly exceeds *cis*-β-carotene in the circulation, whereas an increased proportion of *cis*-β-carotene is observed in peripheral tissues (Stahl and Sies, 1993).

In general, tissue carotenoid concentration directly reflects dietary intake of these compounds, increasing when carotenoids are fed to humans and declining with depletion. For this reason, plasma carotenoids can function as biomarkers of intakes of vegetables and fruit (the major dietary sources), when other influencing variables are considered, and are used as markers of compliance with high-vegetable-diet interventions (Rock *et al.*, 1997).

Although the major focus of most human feeding studies has been on the plasma or serum carotenoid response, concentrations in peripheral tissues also increase as a result of administering these compounds or eating carotenoid-rich foods, presumably owing to an apparent lack of regulatory mechanisms, as has been identified for other dietary factors (e.g., dietary preformed vitamin A). Yellowing of the skin (carotenodermia), which results from continued high doses of carotenoids, is due to the deposition of these compounds in the stratum corneum of the epidermis. Preliminary studies in which stable isotope tracers and a compartmental physiologic mathematical model are used suggest a mean residence time for β-carotene of 51 days in the human biological system, which is longer than predicted from previous (nonisotope tracer) studies (Novotny *et al.*, 1995).

3. METABOLISM

Current knowledge of carotenoid metabolism in humans relates primarily to the relation between these compounds and vitamin A. Only those carotenoids that have at least one unsubstituted β-ionone ring attached to an intact conjugated polyene structure from C-7 to C-15 can be metabolized to retinal, which is then reduced to retinol (Goodwin, 1986). Because of these structural requirements, β-carotene is the most important provitamin A carotenoid, although others (e.g., α-carotene, β-cryptoxanthin) also meet these requirements. The primary mechanism by which β-carotene is metabolized to retinal (which is subsequently converted into retinol) has been believed to be through central cleavage by the cytosolic enzyme β-carotene-15,15′-dioxygenase (Goodwin, 1986). Excentric cleavage, which produces apocarotenol intermediates, also has been demonstrated in human intestinal tissue (Fig. 2) (Krinsky *et al.*, 1993; Wang *et al.*, 1991). The β-apocarotenoic acids corresponding to the β-apo-14′-, -12′-, -10′-, and -8′-carotenals have been isolated and characterized in rat and chicken tissues, and the metabolism of xanthophylls in mammals by excentric cleavage activity also has been suggested (Krinsky *et al.*, 1993).

Much remains to be learned about the metabolism of the various carotenoids and the products of these metabolic reactions in human tissue. Evidence from studies in which human intestinal tissue and rat liver were used indicates that 9-*cis*-β-carotene functions as a precursor for both all-*trans*- and 9-*cis*-retinoic acid (Nagao and Olson, 1994; Wang *et al.*, 1994). Current evidence suggests that carotenoids may be metabolized to compounds other than vitamin A and that these retinoid-like metabolites may affect

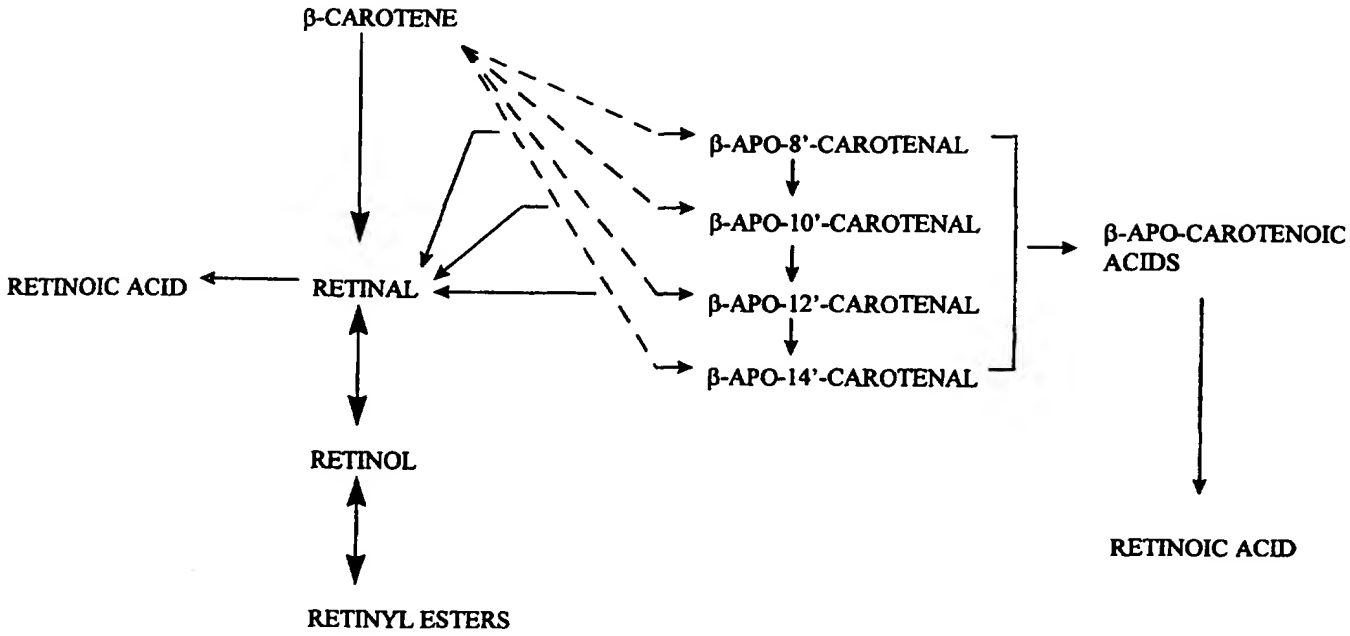

FIGURE 2. Alternative metabolic products of β-carotene. Data from Krinsky *et al.* (1993).

growth regulation and other cellular activities (Krinsky, 1994a). Metabolism of β-carotene to retinal has also been shown to take place in peripheral tissues, such as human adipose tissue, primate lung and kidney and bovine corpus luteum (Schweigert *et al.*, 1988; Wang *et al.*, 1991), in addition to intestinal mucosal cells and hepatocytes.

Provitamin A carotenoids are not converted into retinol unless the need for vitamin A exists, and the conversion efficiency is known to be low. Traditional estimations considered β-carotene to have one-sixth the vitamin A value of an equal amount of retinol, on the basis of an assumption of a 33% average extent of bioconversion of β-carotene into vitamin A and poorer absorption of dietary carotenoids compared with preformed vitamin A (Solomons and Bulux, 1993). Other provitamin A carotenoids were estimated to have one-twelfth the vitamin A value of retinol. Current evidence suggests that these early estimations substantially overestimate the contribution of carotenoids to overall vitamin A status, particularly when improvement in vitamin A stores or the circulating vitamin A concentration is the goal (de Pee *et al.*, 1995; Solomons and Bulux, 1993).

Excretion metabolites from the carotenoids have not yet been identified. The general assumption is that the degradation process for carotenoids and their metabolites is likely to be similar to that of vitamin A and retinoids. Liver cytochromes P450 have been shown to take part in the metabolism of retinol and retinoic acid to polar metabolites (Leo *et al.*, 1989; Raner *et al.*, 1996; Roberts *et al.*, 1992), so the possibility of the involvement of that system in carotenoid metabolism is feasible. Substantial species differences in both the cytochromes P450 system and the usual uptake and tissue exposure to carotenoids make this a difficult area to investigate. Because of the inefficient absorption of these compounds, most of the carotenoids that are ingested will exit the human body in the feces (Rock *et al.*, 1996).

4. DIETARY SOURCES

Carotenoids are present in several types of foods, but most carotenoids in the diet are provided by deeply pigmented vegetables, fruits, and juices. Additionally, smaller amounts can be obtained from milk and foods containing dairy fat, egg yolks, ocean fish and carotenoids added as colorants to foods during processing. Until recently, carotenoid food content data were mainly based on the Association of Official Analytical Chemists method, in which open-column chromatographic procedures separated carotenoids into major classes of compounds, hydrocarbons (carotenes) and oxygen-containing compounds (Simpson and Chichester, 1981). The value produced was for total (rather than individual) carotenoids, and it tended to overestimate the potential vitamin A content of carotenoid-containing plant foods. A recently released database for carotenoid content of fruits and vegetables, based on figures derived from modern HPLC methods, permits increased accuracy in the assessment of carotenoids in the diet (Mangels *et al.*, 1993).

Compared with current food content data for many other nutrients, the analytic data for the carotenoids are still limited in quality, meaning that many of the values used in the development of the database are not necessarily based on documented methodology, appropriate reference material, optimum sample handling or representative sampling of products for analysis (Mangels *et al.*, 1993). Several potential sources of variation in carotenoid content of individual foods have been examined (Beecher and Khachik, 1989;

Khachik *et al.*, 1991). For example, climate is an important determinant of plant carotenoid content: carrots and squash grown in regions with more sunlight have more β-carotene than those grown in areas with less total radiation. The genotype of the plant is another important determinant, with certain cultivars of carrots, peppers, and mangoes having substantially greater amounts of carotenoids than others. Plant maturity also exerts an effect: carotenoid concentrations increase with the ripening of squash and lettuce, and the ratio of β-carotene to β-cryptoxanthin in citrus fruits, such as tangerines and oranges, changes as these fruits ripen. In contrast, soil type and fertility appear to have little effect on the carotenoid content of plants.

Animal food sources of carotenoids, such as dairy products and egg yolk, also exhibit some variability in carotenoid content, which is mainly attributable to differences in animal feed. Season can affect the carotenoid content of milk because cows ingest more from the summer pasture than from winter feeds. In the United States, β-carotene and two other carotenoids that can be synthesized (β-apo-8′-carotenal and canthaxanthin) are approved for use as food colorants in dairy foods, margarine and other dairy replacement products and in some processed foods, such as frozen desserts and baked goods (Gordon and Bauernfeind, 1981). Fewer quantitative data are available for the nonplant food sources of carotenoids, although this lack of data may be less crucial, because these sources appear to be minor contributors of carotenoids in the typical human diet.

Cooking and processing of food affects carotenoid content, with variable degrees of stability evident among the different compounds (Beecher and Khachik, 1989). In general, the most common household cooking methods, such as microwave cooking, steaming or boiling in a small amount of water, do not drastically alter carotenoid content of vegetables. Hydrocarbon (i.e., β-carotene, α-carotene, lycopene) and hydroxylated (i.e., lutein) carotenoids are less susceptible to destruction than are epoxides. Mild heat treatment of yellow-orange vegetables, such as carrots, sweet potato and pumpkin, results in a loss of only about 8–10% of the α- and β-carotene, whereas 60% of the total xanthophylls in green vegetables, such as Brussels sprouts and kale, are lost with similar cooking methods (Beecher and Khachik, 1989). Among the xanthophylls, lutein is the most stable and is more resistant to heat than the others, with a reported reduction of 18–25% from microwave cooking of these vegetables. Drying, extreme heat or extensive cooking time, as occurs with canning at high temperatures for extended periods, results in oxidative destruction of the carotenoids.

Heat treatment also promotes isomerization of the carotenoids in foods, from *trans-* to *cis*-isomeric forms, and the degree of isomerization is directly correlated with the intensity and duration of heat processing. Fresh sweet potatoes, carrots and tomatoes contain negligible quantities of *cis*-β-carotene, whereas the proportion in canned products is approximately 25%, 27% and 47%, respectively (Chandler and Schwartz, 1987).

Vegetables and fruits that are the major sources of carotenoids in the United States diet are shown in Table 2. In general, yellow-orange vegetables and fruits provide most of the dietary β-carotene and α-carotene, orange fruits provide β-cryptoxanthin, dark green vegetables provide lutein and tomatoes and tomato products are the major sources of lycopene in the diet. Typically, a substantial proportion of carotenoid-rich foods that are consumed are in multicomponent or mixed dishes (i.e., soup, stew), a practical detail that must be considered in dietary assessment (Chug-Ahuja *et al.*, 1993; Hankin *et al.*, 1993). On the basis of the new figures for food content, a total intake of 6–11 mg/day of the 5 major carotenoids is the usual amount consumed by adults in the United States. Lycopene is typically the carotenoid consumed in the greatest amount in the diet (>3 mg/day), with β-carotene and lutein intakes slightly lower (approximately 3 and 2–3 mg/day, respectively), with the remainder from β-cryptoxanthin and α-carotene.

Intakes of carotenoids in human diets are more variable than intakes of many other dietary components, so dietary assessment requires special considerations. If the assessment is conducted with the use of dietary records or daily recall, collection of data for many days is necessary to obtain an accurate picture of overall usual intake. A few carotenoid-rich foods usually account for the major proportion of that consumed, and these foods may be eaten episodically rather than daily (Hankin *et al.*, 1993). Studies conducted in the mainland United States do not typically identify a substantial effect of seasonality on serum carotenoid concentrations (Cantilena *et al.*, 1992), probably owing to modern food distribution patterns. In populations or cultural groups that exhibit seasonal patterns in their intake of fruits and vegetables, however, seasonality may be an important determinant of the type and amount of dietary carotenoids

TABLE 2. Major Contributors of Carotenoids in United States Diets

Carotenoid	Food	Amount[1] (μg/100g)
β-Carotene	Carrots, cooked	9771
	Canteloupe, raw	3000
	Broccoli, cooked	1292
	Spinach, cooked	5300
α-Carotene	Carrots, cooked	3723
Lycopene	Tomato, raw	3100
	Tomato sauce, canned	6300
β-Cryptoxanthin	Orange juice	24
	Tangerine, raw	1060
	Peach, raw	42
Lutein	Spinach, cooked	12475
	Broccoli, cooked	1839
	Corn, yellow, cooked	775
	Green beans, cooked	736
	Green peas, cooked	1690

[1]Median value based on modern HPLC methods.
Data from Mangels *et al.* (1993) and Chug-Ahuja *et al.* (1993).

eaten (Olmedilla *et al.*, 1994; Rautalahti *et al.*, 1993). Across different cultures and populations, the usual dietary sources of the major carotenoids can differ a great deal, so food frequency questionnaire or check-list approaches to dietary assessment must be individualized to the cultural or regional group to target the appropriate foods.

5. FUNCTIONS AND BIOLOGICAL ACTIVITIES

As described by Olson (1989) and Krinsky (1994b), biological functions of compounds such as carotenoids should be differentiated from actions or associations. Biological functions are considered to be essential to the well-being of an organism, whereas biological activities are physiologic or pharmacologic responses after administration. Epidemiological studies of carotenoids and disease have been a source of much data suggesting associations, and such associations should be interpreted as hypothesis-generating rather than indicative of a causal relation. As previously reviewed (Bendich, 1993; Krinsky, 1994b; Ziegler, 1989), epidemiologic evidence has strongly linked higher levels of carotenoid intake (or increased circulating concentrations) with reduced risk for many cancers, cardiovascular disease, cataracts and age-related macular degeneration.

5.1. *Biological Functions*

In photosynthetic organisms such as plants, carotenoids are known to have a functional role because of the capability of these compounds to transfer energy in photosynthesis and in photoprotection (Krinsky, 1994b). This function serves to protect cells and tissues from cellular damage related to certain photochemical reactions, such as light-induced photooxidation of chlorophyll and other molecules in plants and microorganisms.

For humans and other animals possessing the enzymatic capabilities for conversion, some of the carotenoids function as vitamin A precursors, and vitamin A is necessary for vision, growth, cellular differentiation, morphogenesis, and several other cellular and physiologic functions (Olson, 1996). The proportion of vitamin A activity contributed by carotenoids in the diet is quite variable across populations and cultures. Individuals and groups consuming hardly any or no dairy products or liver, which are the richest sources of preformed vitamin A, rely to a greater extent on provitamin A carotenoids to meet their dietary vitamin A requirements, when compared with those who consume such foods frequently.

5.2. *Biological Activities*

The many biological activities of carotenoids observed in various basic and experimental research studies have been reviewed previously (Krinsky, 1993, 1994a,b). Carotenoids are efficient quenchers of singlet oxygen and can directly scavenge free radicals (Krinsky, 1993; Stahl and Sies, 1993). Vitamin A, in comparison, is a relatively poor antioxidant. Some variability in antioxidant activity among the carotenoids has been observed *in vitro*; for example, lycopene exhibits superior antioxidant capability when compared with β-carotene and lutein (DiMascio *et al.*, 1991). The *in vivo* antioxidant actions of carotenoids have been demonstrated in many studies (Krinsky, 1993), in which the effect is mainly evident in the inhibition of lipid peroxidation in the presence of these compounds. Carotenoids also participate in cooxidation reactions, such as those in which a carotenoid and a polyunsaturated fat undergo cooxidation. *In vitro* studies using soybean lipoxygenase have shown that β-carotene may inhibit the production of lipid peroxyl radical products of the lipoxygenase reaction in this manner (Canfield and Valenzuela, 1993).

The antioxidant effect of carotenoids in LDL has been suggested to be the biological link between higher levels of dietary carotenoids and reduced risk for cardiovascular disease. However, *in vivo* evidence to support a relation between carotenoids and LDL oxidation has not been consistent (Krinsky, 1994b; Rock *et al.*, 1996). A biological explanation may be that, compared with the carotenoids, many more molecules of another important lipid-soluble antioxidant, α-tocopherol, are present in LDL (DiMascio *et al.*, 1991), so the antioxidant activities of carotenoids are of lesser importance in the prevention of LDL oxidation. However, the presence of lutein and zeaxanthin as specific macular pigments may have particular clinical relevance to the association between these dietary carotenoids and age-related macular degeneration, which appears to be related to antioxidant defenses in that tissue (Seddon *et al.*, 1994).

Anticarcinogenic activities have been demonstrated for both provitamin A and nonprovitamin A carotenoids in several animal models (Krinsky, 1993). In several types of cell cultures, carotenoids have been shown to promote inhibition of growth and of malignant transformation (Krinsky, 1994a). In human mammary epithelial cells, morphologic changes suggesting differentiation were observed to accompany a reduced proliferative capacity in response to both β-carotene and canthaxanthin, a nonprovitamin A carotenoid (Rock *et al.*, 1995). The prevention of malignant transformation in mouse fibroblast cells was found to be mediated by increased gap-junction communication between cells, which was subsequently shown to be due to the up-regulation of the connexin 43 gene (Bertram, 1994; Zhang *et al.*, 1991) and to be unrelated to the antioxidant capacities of the compounds. Prevention of neoplastic transformation and modulation of gene expression by carotenoids, and modulation of differentiation similar to the effect of retinoids, has also been demonstrated in human keratinocytes (Bertram and Bortkiewicz, 1995). These effects were observed in response to both provitamin A and nonprovitamin A carotenoids, with the use of concentrations in the cell culture medium that are achievable by increased dietary intake. Thus, although much of the initial interest in the biological link between carotenoids and carcinogenesis was focused on antioxidant activities, current evidence instead supports the likelihood that the major anticarcinogenic effects of carotenoids relate to cellular growth regulation, similar to the effects of retinoids. Rather than intact

carotenoid molecules, retinoid-like metabolites produced locally in tissues are likely to be the active agents (Krinsky, 1994a).

As previously reviewed by Bendich (1991), administration of provitamin A and nonprovitamin A carotenoids to laboratory animals and β-carotene supplementation to humans have been shown to enhance various indices of immune function when compared with a control feeding regimen or placebo. As an example, both β-carotene and 13-*cis*-retinoic acid were observed to produce significant, but different, effects on immune cell populations in patients enrolled in clinical trials (Prabhala *et al.*, 1991), with β-carotene producing an increase in the percentage of cells expressing natural killer cell markers and a smaller effect on T-helper cells. Among male smokers in another clinical trial, no effect on lymphocyte subsets, but a 12% higher phytohemagglutinin-induced lymphocyte proliferation, in those provided β-carotene (versus controls) was observed (van Poppel *et al.*, 1993). In comparison, extensive evaluation of immune response indicators in women who had adequate vitamin A status and were fed carotenoid depletion and repletion diets failed to demonstrate a relation between immune system indexes and carotenoid status (Dauda *et al.*, 1994). The potential clinical relevance of a relationship between carotenoids and immune responses is illustrated by the observation that β-carotene supplementation could prevent the photosuppression of delayed-type hypersensitivity in men (Fuller *et al.*, 1992), and UV light exposure both reduces immune response and increases risk for skin cancer. Much remains to be learned about the mechanism of action of carotenoids on immune parameters and how this activity may relate to disease risk and treatment.

6. CAROTENOIDS IN CHEMOPREVENTION AND TREATMENT

6.1. *Carotenoids in the Treatment of Disease*

β-Carotene supplementation is recognized as an efficacious treatment modality in the management of the genetic photosensitivity disease erythropoietic protoporphyria, with a strategy that is based on the function of carotenoid pigments in plants and microorganisms (Mathews-Roth, 1993). Patients with erythropoietic protoporphyria have defective ferrochelatase, the enzyme that inserts iron into protoporphyrin to produce heme, which results in an accumulation of protoporphyrin. Without treatment, patients experience itching, burning, and ulceration of skin upon exposure to light. Adults with this genetic disease are started at a level of 180 mg/day, which may be increased to as much as 300 mg/day, with lower dosages prescribed for children and teenagers. Although the blood and stool porphyrin levels are not affected, this treatment strategy has been shown to increase the ability to tolerate sunlight exposure by a factor of three or more in the majority of patients who have been studied. Presumably, the accumulation of carotenoids in the tissue prevents photosensitization by the porphyrins, which are similar to the porphyrin group of chlorophyll. The use of high-dose β-carotene has also been investigated in the treatment of other photosensitivity diseases and in the prevention of sunburn, but with somewhat more variable success rates (Mathews-Roth, 1993).

6.2. *Carotenoids in Chemoprevention*

On the basis of the substantial amount of epidemiologic evidence linking carotenoids to risk for chronic disease (especially cancer) that had accumulated by the 1980s and the strength of the basic and experimental supportive evidence, several large clinical trials were initiated at that time to test the effect of intervention (Ziegler, 1993). Notably, these studies were planned and implemented with very limited knowledge of the pharmacokinetics and metabolism of these compounds. In these trials, β-carotene supplementation at varying doses (typically 20–50 mg/day) was the planned intervention. Among the carotenoids typically provided by the diet, β-carotene is the only carotenoid commercially available as a synthetic product to be administered to humans, with the others either produced in limited amounts for agricultural or laboratory use or isolated from foods by labor-intensive procedures. Clinical trials traditionally rely on single or very few nutrients in the assessment of cancer prevention (Greenberg, 1993) and, of necessity, usually must target very high-risk groups (or cancer recurrence) rather than aim to examine effects on primary prevention.

Six major chemoprevention trials with β-carotene supplementation have now been completed, with results reported in the literature (Blot *et al.*, 1993; Greenberg *et al.*, 1990, 1994; Hennekens *et al.*, 1996; Omenn *et al.*, 1996b; The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). Supplementation with 50 mg β-carotene/day for 5 years had no effect on the occurrence of new basal- or squamous-cell carcinoma in well-nourished patients who had had skin cancer previously (Greenberg *et al.*, 1990), although a 12-year latency period for these cancers must be considered. In another large multicenter trial (Greenberg *et al.*, 1994), β-carotene (25 mg/day) with or without vitamin C (1 g/day) and α-tocopherol (400 mg/day) for 5–8 years was not found to reduce the occurrence of colorectal adenoma in patients who had a prior history of adenomas.

In a large Finnish trial (The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994), intake of 20 mg/day β-carotene for 5–8 years was found, surprisingly, to be associated with an 18% increased incidence of lung cancer in male smokers. As in the earlier observational studies, however, both dietary and serum β-carotene at baseline were found to be inversely related to risk of lung cancer during the trial. Overall findings were very similar in the multicenter Carotene and Retinol Efficacy Trial (CARET), in which 30 mg β-carotene and 25,000 IU vitamin A were administered daily to smokers, former smokers, or persons exposed to asbestos, which was halted because of an apparent 28% increased lung cancer risk in the treat-

ment group (Omenn *et al.*, 1996b). Another similarity of CARET to the Alpha-Tocopherol, Beta Carotene (ATBC) Study was the finding that participants with higher serum β-carotene concentrations at entry into CARET had fewer subsequent lung cancers, regardless of treatment group assignment. Further analysis of these two trials provide interesting details regarding the surprising finding of increased overall lung cancer risk in association with β-carotene supplementation, which was the opposite result than was expected. Subgroup analysis of the ATBC Study revealed that the adverse effects of supplemental β-carotene were found among participants who smoked ≥20 cigarettes/day or who reported higher levels of alcohol intake (Albanes *et al.*, 1996). These relationships between smoking status, alcohol consumption, and increased risk associated with β-carotene supplementation were also observed in subgroup analysis of the CARET Study: participants who were former (versus current) smokers and who consumed lower amounts of alcohol did not have increased lung cancer risk in association with supplementation (Omenn *et al.*, 1996a).

The Physician's Health Study, a large, 12-year study in which male physicians who were primarily nonsmokers took 50 mg β-carotene supplements every other day, reported neither benefits nor adverse effects of the intervention on malignant neoplasms and cardiovascular disease (Hennekens *et al.*, 1996). In Linxian, China, administration of a combination of 15 mg β-carotene, 50 μg selenium, and 30 mg α-tocopherol per day for 5–6 years reduced the incidence of cancers of the upper gastrointestinal tract compared with other vitamin and mineral combinations in a large population of undernourished adults (Blot *et al.*, 1993).

Thus, results from the majority of clinical trials reported to date are not supportive of the strategy of using β-carotene supplementation as a means to reduce cancer and cardiovascular disease rates. In fact, many questions have been raised about the wisdom of conducting these trials when so many of the biological characteristics of the carotenoids that should be considered in conducting such trials are unknown, such as the appropriate chemical and isomeric form, dosage, possible interactions, optimal timing for intervention, and optimal study duration (Erdman *et al.*, 1996; Rock *et al.*, 1996). The epidemiologic link between antioxidant micronutrients and disease is based primarily on evidence pertaining to dietary intake or on blood levels that are mainly the result of food choices rather than supplements. The serum concentrations of β-carotene that are achieved with supplementation are substantially higher than those associated with diet, even when compared with concentrations achieved with the consumption of a carotenoid-rich diet (Mayne *et al.*, 1996; Rock *et al.*, 1997), which may contribute to the adverse effects observed in some subgroups in the ATBC and CARET studies. No biologically active agent administered in high doses can be considered risk free for all people, even if the agent is a normal constituent of the food supply.

Several clinical trials in progress are investigating the efficacy of a carotenoid-rich diet intervention, for example, in promoting the reversal of cervical dysplasia and in the prevention of breast cancer recurrence (Rock *et al.*, 1997; Pierce *et al.*, 1997). A role for carotenoids in the prevention of breast cancer recurrence has been suggested by observations that higher levels of carotenoid intakes at diagnosis are associated with greater likelihood of survival in several epidemiologic studies (Ingram, 1994; Jain *et al.*, 1994; Rohan *et al.*, 1993). An advantage of a diet intervention approach is that it may be considered a risk-free strategy because, to date, no adverse effects have been associated with consuming more carotenoids from food sources. In addition, benefits may be gained from such an approach, even if other biologically active components of a carotenoid-rich diet (rather than the carotenoids themselves) are found to be the truly active agents.

6.3. *Safety and Toxicity*

As a food additive, β-carotene is in the United States Food and Drug Administration category of foods generally recognized as safe for use as a colorant and as a dietary supplement (Diplock, 1995). This categorization is supported by numerous studies in several model systems to determine possible mutagenicity, embryotoxicity, and teratogenicity of carotenoids (Bendich, 1988). Until the results of the ATBC and CARET studies suggested an increased risk for lung cancer associated with β-carotene supplementation among heavy smokers, β-carotene was considered to be completely safe when administered orally to humans, as reviewed by Bendich (1988) and Diplock (1995). Mechanisms to explain the observed associations among heavy smoking, alcohol consumption, β-carotene supplementation, and lung carcinogenesis in the two clinical trials are unknown, although several hypotheses have been proposed (Erdman *et al.*, 1996; Mayne *et al.*, 1996).

Excessive intake of β-carotene or other provitamin A carotenoids does not promote hypervitaminosis A. Hypercarotenemia, or yellowing of the skin, occurs with high doses of β-carotene (>30 mg/day) administered over extended periods of time (Bendich, 1988) and is a benign condition that spontaneously resolves when the dosage is reduced or discontinued. A report of possible leukopenia in association with the consumption of large quantities of fresh carrot juice could not be confirmed in larger series or trials (Bendich, 1988; Mathews-Roth, 1993). The use of large doses of canthaxanthin, sold in some European countries as a tanning aid, has been associated with the reversible deposition of pigmented granules in the retina and some loss in night vision (Arden and Barker, 1991). High-dose β-carotene supplementation has not been found to result in the development of such granules (Mathews-Roth, 1993).

7. CONCLUSIONS

Carotenoids may be considered micronutrients, because of their relationship to vitamin A, and also phytochemicals or biologically active plant constituents. Vitamin A deficiency

remains a major nutritional problem in most economically disadvantaged areas of the world, where populations generally rely on dietary carotenoids to meet vitamin A needs. In developed nations, biological activities relating to the prevention and treatment of disease are of great interest because the amounts of these biologically active compounds in tissue can be modulated by food choices. The techniques and methodologies necessary to investigate the carotenoids in the human biological system are relatively newly developed, and have resulted in an enormous expansion in knowledge in the past two decades. However, many of the crucial aspects of metabolism and the molecular basis for the biological activities of carotenoids are still unknown, and this lack of knowledge constrains the ability to examine the role of carotenoids in health and disease. Continued research in this area is likely to stimulate additional and better-refined strategies for intervention, with both clinical and public health applications.

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